DATA EVALUATION RECORD

IMIPROTHRIN [S-41311]

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY – RAT
OCSPP 870.3700a [§83-3a]; OECD 414

MRID 49192902

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OCSPP 870.3700a [§83-3a]; OECD 414.

PC CODE: 004006 **DP BARCODE:** D414384

TEST MATERIAL (PURITY): Imiprothrin (92.9% a.i.).

SYNONYMS: S-41311.

CITATION: Ohtsuka, T. (1992). Reproduction study of S-41311 in rat with administration during the period of fetal organogenesis. Panapharm Laboratories Co., Ltd., Safety Assessment Laboratory, Kurisaki-machi 1285, Uto-shi, Kumamoto 869-04, Japan. Laboratory report number 49111, August 26, 1992. MRID 49192902. Unpublished.

SPONSOR: Sumitomo Chemical Company, Ltd., 5-33 Kitahama, 4-chome, Chuo-ku, Osaka, 541, Japan.

EXECUTIVE SUMMARY: In this non-standard, combination developmental toxicity and reproduction toxicity study (MRID 49192902), S-41311 (Imiprothrin; 92.9% a.i.; Lot No. Y-011001) was administered to 36 Charles River Sprague-Dawley female rats/dose by gavage in corn oil at dose levels of 0, 50, 200, or 600 mg/kg bw/day on gestation days (GD) 6 through GD 17. On GD 20 (developmental toxicity segment of the study), 24 F0 dams per group were sacrificed, and developmental parameters were evaluated as follows: the numbers of corpora lutea, implants, early resorptions, late resorptions, and live and dead fetuses were recorded; the live fetuses were sexed, weighed, and observed for external anomalies including the oral cavity; about one-third of the live fetuses in each litter were examined for visceral anomalies, including the head; the remaining two-thirds of the fetuses in each litter were subjected to skeletal examination; and the placentae were weighed.

The remaining ~12 F0 dams per group were allowed to litter and raise their (F1) offspring. The F1 offspring matured and were mated at 12 weeks (were not treated with imiprothrin). There are two F1 segments). Segment 1, a reproductive study, is reported in Appendix I of this DER. As part of the reproduction study, skeletons were stained at weaning, postnatal differentiation of the offspring was studied, and function tests were carried out; these data are also included in Appendix I. Segment 2, a developmental toxicity study (F2), is reported in Appendix II of this DER.

Maternal (FO) toxicity (developmental phase): Tremors and convulsions were observed in most FO dams approximately 5 minutes after dosing initially (GD 6-9), with prone position in about half of the dams, and staggering gait in a few dams. Body weights were not adversely affected during gestation. **The F0 maternal lowest-observed-adverse-effect level (LOAEL) in rats treated with imiprothrin on gestation days 6-17 is 600 mg/kg bw/day, based on ~~mortality and~~ clinical signs (tremors and convulsions). The F0 maternal no-observed-adverse-effect level (NOAEL) is 200 mg/kg bw/day.**

Developmental toxicity: There were no treatment-related increases in fetal deaths/resorptions or malformations. Significantly reduced body weight was observed in male and female fetuses in the 600 mg/kg bw/day group (6% less than controls). The 600 mg/kg bw/day group also had decreases in the ossification percentages of the fifth and sixth sternebrae and the proximal phalanges of the forelimb, both left and right. In the morphological examination of the viscera, the fetal incidence of thymic remnant in the neck was significantly increased in the 600 mg/kg bw/day group (18.9% vs. 0% of control fetuses; $p<0.01$). There was a dose-related increase in the fetal incidence of skeletal variations at all dose levels, with the incidences at 200 and 600 mg/kg bw/day being statistically significant ($p<0.01$). In particular, there was a significantly ($p<0.01$) higher incidence of skeletal variations (lumbar ribs) in the 200 and 600 mg/kg bw/day groups than in the control (15.67%, 19.82%, 47.51%, and 87.95% of fetuses affected, in ascending group order). The litter incidence of bilateral lumbar ribs at 50 mg/kg bw/day (34.8%) was comparable to the concurrent control (30.4%), and the litter incidences at the 200 mg/kg bw/day (82.6%) and 600 mg/kg bw/day (95.8%) were outside of the litter incidence of the control (30.4%). **The developmental toxicity LOAEL in rats treated with S-41311 (imiprothrin) on GD 6-17 is 200 mg/kg bw/day, based on an increased incidence of unilateral and bilateral lumbar ribs. The developmental toxicity NOAEL is 50 mg/kg bw/day. At 600 mg/kg/day, there also was a decrease in fetal body weight and increased incidences of thymic remnant in the neck, pre-sacral vertebrae, and split vertebral body, and reduced ossification of 5th and 6th sternebrae and proximal forelimb phalanges.**

The developmental toxicity study in the rat is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OCSPP 870.3700) in the rat.

Additional/supplementary findings from the non-guideline portion of the study:

Maternal toxicity: In the F0 females that were allowed to litter and raise their young, there were two treatment-related deaths (GD 9 and GD 12) at 600 mg/kg bw/day. Tremors and convulsions were observed in most FO dams approximately 5 minutes after dosing initially (GD 6-9), with prone position in about half of the dams, and staggering gait in a few dams. Body weights were not adversely affected during gestation. In the F1 parental females sacrificed on gestation day 20, no significant maternal effects were noted. T**he F0 maternal toxicity LOAEL in rats treated with imiprothrin on gestation days 6-17 and allowed to deliver their young is 600 mg/kg/day, based on maternal mortality and clinical signs (tremors and convulsions), which occurred during the first 3 days of dosing. The F0 maternal toxicity NOAEL is 200 mg/kg bw/day. The maternal toxicity NOAEL of the F1 parental animals who were dosed *in utero* and during lactation but not directly after weaning or during their own mating and gestation phases is 600 mg/kg/day, the highest dose tested.**

Offspring toxicity: The number of stillborn males and females (6, plus 1 cannibalized) was increased at 600 mg/kg bw/day compared to the control (1). The number of implantations, number of live offspring, and sex ratio showed no dose-related changes. No dose-related differences were observed in the growth of offspring to weaning. No dose-related differences were observed in postnatal differentiation or in function test results. At weaning, one male and one female from each litter were stained and examined for skeletal alterations. In the 600 mg/kg bw/day group, there was a significant increase in the number of offspring with bilateral lumbar ribs. **The offspring LOAEL following *in utero* exposure to imiprothrin on gestation days 6-17 is 600 mg/kg bw/day, based on an increase in the number of stillborn and an increased number of weanlings with bilateral lumbar ribs. The offspring NOAEL is 200 mg/kg bw/day.**

Reproductive toxicity: In the F1 parental animals who were dosed *in utero* and during lactation but not directly after weaning or during their own mating and gestation phases, there were no dose-related changes in copulation index, pregnant index, or the duration of mating.

Developmental toxicity: In the F1 dams/F2 fetuses, there were no dose-related changes in the numbers of corpora lutea, implants, pre-implantation loss, early resorptions, late resorptions, dead fetuses, live fetuses, sex ratio, body weight, or placental weight of live fetuses. The F2 fetuses, who were not exposed during gestation, showed no dose-related external findings (skeletal and visceral assessments were not performed on these fetuses).

The reproductive/developmental/offspring toxicity portion of this study in the rat is classified as **Acceptable/Non-guideline**. It is non-guideline due to the lack of exposure of the F1 parental animals during their pre-mating, mating, and gestation periods to the test material. Additionally, there are reporting deficiencies, in particular the omission of litter data, organ weight data, and the inadequacy of the histopathology report. However, it provides supplemental information on the reproductive/developmental/offspring outcome following gestational/lactational exposure of the F0 parental animal.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: S-41311

Description:	Yellow or pale yellow viscous liquid,
Lot/batch #:	Lot No. Y-011001
Purity:	92.9 % a.i.
Compound stability:	Stable under refrigeration (8 days) and continuous 6 hours at room temperature
CAS #of TGAI:	Not available
Structure:	Not available

2. Vehicle and/or positive control: Distilled corn oil J.P. (Lot No. M59600B and L33995A, Kishida Chemical Co., Ltd.)

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley (Crj:CD)	
Age/weight at study initiation:	Females: approximately 12 weeks of age; 237.8-287.0 g Males: approximately 12 weeks of age; 383.2-453.3 g	
Source:	Oriental Yeast Industry Co., Ltd.	
Housing:	Housed individually in stainless steel hanger cages except during mating; females housed individually in polycarbonate cages with bedding during gestation	
Diet:	Pelleted diet MF (Oriental Yeast Industry Co., Ltd.) *ad libitum*	
Water:	Well water (+ 2 ppm sodium hypochlorite) *ad libitum* via automatic watering system or bottles	
Environmental conditions:	**Temperature:**	24±2°C
	Humidity:	55±10%
	Air changes:	13/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	About 2 weeks	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: June 24, 1991; End: Aug. 26, 1992

2. Mating: Sexually mature females were caged overnight with sexually mature males of the same age, strain, and source (1 male: 1 female). Confirmation of mating was determined by the presence of sperm in the vaginal smear or the presence of a copulatory plug. The day on which evidence of mating was observed was designated as gestation day (GD) 0.

3. Animal assignment: Assignment to groups was based on animal weight at GD 0. Each pregnant rat weighing 237.8 to 287.0 g was assigned to a group so as to make the body weight at GD 0 equivalent in all groups. Assignment was not random. The dose groups are indicated in Table 1. The manner in which the pregnant F0 females were allocated for the prenatal developmental or reproductive/postnatal toxicity portions of the study was not mentioned in the study report. The manner in which the animals to form the F1 parental generation were selected also was not described in the study report. It is noted that the F1 parental generation was not dosed with test material.

TABLE 1. Animal assignment

Treatment group	Control	Low-Dose	Mid-Dose	High-Dose
Dose (mg/kg bw/day)	0	50	200	600
F0 mating:				
No. females mated	36	36	36	36
No. pregnant females	35	35	34	36
Developmental toxicity	23	23	23	24
Reproduction	12	12	11	9
Number of deaths	0	0	0	3
F1 mating:				
No. females mated	24	24	22	18
No. pregnant females	24	22	22	16

4. **Dose selection rationale:** The dose levels were selected based on the results from a preliminary developmental toxicity study where doses of 0, 400, 600, 800, or 1000 mg/kg bw/day were given. The route of administration and the days of treatment are not stated but in the study report there is a reference to an unpublished report by Fuchigami et al. (1992). At 600 mg/kg bw/day and above, clonic convulsion, exophthalmia, incontinentia urinae, and prone position were observed. At 800 and 1000 mg/kg bw/day, animals died (one at 800 mg/kg bw/day and four at 1000 mg/kg bw/day), and reduced body weight, staggered gait, bradypnea, tremor, lacrimation, and loose stool were observed. Decreased food consumption was observed in all treated groups during the early stages of administration. There were no dose-related findings in the uterus. Decreased fetal body weight was observed in the 800 and 1000 mg/kg bw/day groups. Based on these results, 600 mg/kg bw/day was considered tolerable to pregnant rats to provide sufficient numbers of offspring for evaluation. The other two doses were determined as 200 and 50 mg/kg bw/day.

5. **Dosage preparation and analysis:** S-41311 was dissolved in corn oil J.P. and 1, 4, and 12% w/v solutions were obtained. After S-41311 was softened in a hot water bath, it was instilled in a measuring cylinder on a balance with a pipette. Corn oil was added while warming in the water bath. The SOP's for mixing were not available. The prepared test solutions were divided and poured into brown vials for daily use. Test material-vehicle mixture was prepared once or twice per week and stored under refrigeration until administration, when it was used after warming in a water bath. Stability analysis prior to the study indicated that 3% and 25% solutions of the test material in the vehicle were stable for at least eight days under refrigeration. Concentrations of test solutions were analyzed at the first and last administrations, and samples of a 1% test solution from the initial batch were analyzed for stability. There is no indication that homogeneity was evaluated.

Results:
Homogeneity analysis: Not done.

Stability analysis: After 8 days in a refrigerator and continuous 6 hours at room temperature, the measured concentrations were 96.4 to 101.4% of their initial (day 0) values.

Concentration analysis: The measured concentrations were 98.8 to 101.3% of nominal at first administration and 97.6 to 100.4% of nominal at last administration. Variations were within 5% deviation from the nominal concentration, which is within acceptable limits.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered in corn oil once daily by gavage on gestation days 6 through 17 in a volume of 5 mL/kg of body weight/day. Dosing was based on body weight on gestation day 6. The control group was treated with corn oil by the same method for the same period.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs twice daily (morning and afternoon) during the administration period and at least once daily during the remainder of the study. For the F0 dams assigned to natural delivery, clinical observations were made on delivery and lactation. Body weight and food consumption data were recorded on gestation days 0, 4, and 6-20, and on lactation days 0, 4, 7, 10, 14, 17, and 21. Both F0 dams for cesarean section and after weaning were sacrificed by exsanguination from the outer iliac artery under deep anesthesia by ether. For developmental toxicity, dams were sacrificed on gestation day 20. The ovaries and uterus were removed, and the numbers of corpora lutea, implants, early resorptions, late resorptions, and live and dead fetuses were recorded.

2. **Fetal evaluations:** The live fetuses were sexed, weighed, and observed for external anomalies including the oral cavity, and the placenta was weighed. About one-third of the live fetuses in each litter were fixed in Bouin's solution and visceral anomalies were observed, for the head and abdomen, with a razor blade, according to the technique by Wilson (1965), and with the micro-dissection method of Nishimura (1974) for the chest. The remaining two-thirds of the fetuses in each litter were fixed in 70% ethanol, stained with Alizarin Red S (Staples et al., 1964) and examined for skeletal anomalies, variations, hypoplasia, and the progress of ossification. Fetuses with external anomalies were fixed in 10% neutral buffered formalin. The method of selection of fetuses for visceral or skeletal examination was not reported.

D. DATA ANALYSIS:

1. **Statistical analyses:** Mean values and standard deviations were calculated for each group for body weight, food consumption, number of implantation sites, litters, vertebral bodies, vertebral arch, corpora lutea, implants, and live fetuses. Bartlett's test was used to compare the variance among groups of data. When groups were accepted to be homogeneous, analysis of variance (ANOVA) was used for comparison of groups of data. When significant difference was shown among the groups, Dunnett's test (N equal to N) or Scheffe's test (N not equal to N) was used to determine the difference between control and treated groups. When

the groups of data were heterogeneous in Bartlett's test, the Kruskal-Wallis non-parametric ANOVA was used. When there was a significant difference among the groups, a Dunnett-type test or Scheffe's test was used. Mating index, pregnancy index, and sex ratio of live fetuses were analyzed by chi-square test. External anomalies, visceral anomalies, skeletal anomalies, skeletal variations, delayed ossification, progress of ossification, pre-implantation loss, early resorptions, late resorptions, and dead fetuses were analyzed by Wilcoxon's test. In each test, a level of $p<0.05$ was considered statistically significant. Values of F1 fetuses were treated with each litter as a unit.

2. **Indices:** The following indices were calculated:

Gestation Index = (No. females with live newborns/No. pregnant females) X 100

Pre-implantation loss was calculated by the investigator, but the formula was not reported in the study report.

The reviewer calculated the following indices:

Sex ratio (Mean % males) = (No. male fetuses/Total No. live fetuses) X 100

Post-implantation loss (%) = (No. live fetuses/No. implants) X 100

3. **Historical control data:** A small amount of historical control data were provided to allow comparison with concurrent fetal controls. Data for the occurrence of lumbar ribs (1.63-18.61%), split vertebrae (0-1.85%), pre-sacral vertebrae (0-2.6%), and thymic remnants (0-6.3%) were stated. However, no information was provided concerning the number of litters or the number of fetuses the data were based on. Also, the name of the laboratory is different from the one that did this study. On page 28 of the study report, there is a reference to fetal weight obtained from "our lab" but there is no indication of the number of litters or fetuses the data are based on. Male fetal weight was given as 3.5-3.79 g, and female fetal weight was given as 3.38-3.65 g.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** No clinical anomalies were observed during gestation in animals in the control, 50, and 200 mg/kg bw/day groups. Tremor and clonic convulsion were observed approximately 5 minutes after dosing in the early stages of the administration period (Gestation Days GD 6-10) in nearly all dams in the 600 mg/kg bw/day group (Table 2) and continued in 1-2 throughout the dosing period. Animals of the 600 mg/kg bw/day group also exhibited prone position, exophthalmia, and staggering gait, and there were a few animals with salivation, lateral position, incontinentia urinae, hypoactivity, soiled perinaris, and loose stool. Three animals died from the 600 mg/kg bw/day group, one on each of gestation days 9, 12, and 13 (all from the females assigned to the reproduction phase); the death on GD 13 was attributed to a gavage accident.

Table 2. Clinical Signs in F0 Dams at 600 mg/kg/day.												
GD[A]	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
n=	36	36	36	36	35	35	35	34	33	33	33	33
Tremor	5	20	10	10	5	1	1	2	1	1	2	1
Clonic convulsion	2	19	9	10	5	3	2	1	0	1	1	1
Prone position	2	19	9	11	5	3	1	1	0	1	1	1
Staggering gait	0	2	2	1	2	1	0	0	0	0	0	0
Lateral position	0	0	0	1	1	1	0	1	0	0	0	0

Data from page 44 of the study report (MRID 49192902); [A]gestation day

2. **Body weight:** Body weight data are summarized in Tables 3a and 3b. Mean absolute body weights of the treated groups were similar to those of controls throughout gestation. A statistically significant decrease was seen in the 600 mg/kg bw/day group on GD 8 but was only 4% in magnitude. Body weight gain (relative to GD 6 absolute body weight) was significantly reduced in the 200 mg/kg group on gestation days 8 and 10-12, and in the 600 mg/kg bw/day group on gestation days 8-13 and 15. A dose-related decrease in body weight gain was observed in all treated groups during the treatment period, which returned to normal after treatment.

TABLE 3a. Mean maternal body weight (grams) [a]

Interval	Dose in mg/kg bw/day			
	Control	**50**	**200**	**600**
GD 6	297.6±10.9	297.5±9.9	295.0±12.6 (34)	296.2±13.3 (33)
GD 7	297.4±11.7	296.9±10.3	294.1±13.0 (34)	293.1±13.8 (33)
GD 8	302.0±12.7	301.4±10.4	295.6±13.9 (34)	292.7±14.9* (33) ↓4%
GD 9	304.6±13.3	305.8±9.6	300.1±13.2 (34)	296.2±16.7 (33)
GD15	339.3±15.5	338.4±12.4	334.2±15.3 (34)	333.5±17.0 (33)
GD 16	348.8±16.0	348.4±13.5	345.1±14.0 (34)	344.6±18.2 (33)
GD 20	405.1±23.1	407.1±16.4	404.7±20.3 (34)	402.4±20.4 (33)
LD 0	297.2±15.8 (12)	298.7±19.7 (12)	293.5±13.4 (11)	295.8±14.7 (9)
LD 21	323.4±18.1(12)	319.0±18.0 (12)	317.6±12.2 (11)	330.6±14.8 (9)

[a] Data from Table 2, page 47 of the study report; n=35 (unless)

TABLE 3b. Mean maternal body weight gain (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (35)	**50 (35)**	**200 (34)**	**600 (33)**
Pretreatment:				
Days 0-6 [b]	30.6	30.4	29.4	28.8
Treatment:				
Days 6-11	19.7±5.7	18.7±4.2	15.6±5.1 * (-21) [c]	12.8±10.1 * (-35)
Days 6-17	64.3±9.0	63.7±7.8	62.2±7.2	58.8±10.2 (-9)
Post-treatment:				
Days 17-20 [b]	43.3	45.9	46.7	46.5
Days 0-20 [b]	138.1	140.0	138.2	135.0

[a] Data obtained or derived from pages 47-48 in the study report. During gestation, developmental and reproductive toxicity data were combined by the investigator. At 600 mg/kg bw/day, 36 was the initial number of dams; 3 died.

[b] Calculated by the reviewer, using the group means; not analyzed statistically.

c Numbers in parentheses equal percent different from control; calculated by reviewer.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

3. Food consumption: Significantly reduced food consumption was seen in the 50 mg/kg bw/day group on gestation day 12. Food consumption was significantly reduced in the 200 mg/kg bw/day group on gestation days 7, 8, 10, and 12 (11%, 17%, 9%, and 11% less than controls, respectively; $p<0.05$ or $p<0.01$), and in the 600 mg/kg bw/day group on gestation days 7, 8, 9, and 10 (-35%, -37%, -24%, and -17%, respectively; $p<0.01$). Food consumption during pretreatment (days 0-6), treatment (days 6-17), post-treatment (days 17-20), and during gestation (days 0-20) are summarized in Table 4 below. During treatment and during total gestation time, there was a dose-related decrease in food consumption.

TABLE 4. Mean maternal food consumption (g/day/rat) [a]

Interval		Dose in mg/kg bw/day (# of Dams)			
		Control (35)	50 (35)	200 (34)	600 (33)
Pretreatment:	**Days 0-6 [b]**	19.2	19.3	19.3	18.8
Treatment:	**Days 6-17 [b]**	19.0	18.5	18.0	17.6
Post-treatment:	**Days 17-20 [b]**	21.9	21.8	22.2	23.0
Gestation:	**Days 0-20 [b]**	19.5	19.1	18.8	18.6

a Data taken or derived from page 50 in the study report. During gestation, developmental and reproductive toxicity data were combined by the investigator. At 600 mg/kg bw/day, 36 was the initial number of dams; 3 died.
b Calculated by the reviewer as a mean of means; not analyzed statistically.
c Numbers in parentheses equal percent different from control; calculated by reviewer.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

4. Gross pathology: There was no dose-related increase in any of the pathology observations. A blackish coloration and enlargement of the spleen were observed in one dam of the 600 mg/kg bw/day group. A diaphragmatic hernia was observed in one dam each in the control, 200, and 600 mg/kg bw/day groups. A white macule was observed in the liver of one dam of the 600 mg/kg bw/day group. Pelvis dilatation and pitted kidney were observed in two dams of the control group. No gross pathology was observed in any dams of the 50 mg/kg bw/day group.

5. Cesarean section data: Cesarean section data are summarized in Table 5. Significantly reduced body weights of male and female fetuses were observed in offspring of the 600 mg/kg bw/day group. Body weights of fetuses in the 50 and 200 mg/kg bw/day groups were similar to those of controls. Decreased fetal body weight (↓6%) was observed in both sexes at 600 mg/kg/day and is considered treatment-related. Although the number of fetuses per litter was slightly greater (15.08) at 600 ppm compared to the control (14.65), a similar increase in litter size (15.08) was observed at 50 ppm with no body weight deficit. The mean fetal body weight for both sexes is lower than that of the historical control (male range 3.5-3.79 g; female range 3.38-3.65 g). There were no differences in the treated groups in the numbers of corpora lutea, implants, pre-implantation loss, total dead fetuses, early resorptions, late resorptions, dead fetuses, live fetuses, sex ratio, and placental weight of live fetuses.

TABLE 5. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	50	200	600
# Animals pregnant	23	23	23	24
Maternal wastage				
No. died	0	0	0	0
No. aborted	0	0	0	0
No. premature delivery	0	0	0	0
Total No. corpora lutea	375	390	383	393
[Corpora lutea/dam ± SD]	[16.30±1.26]	[16.96±2.12]	[16.65±1.64]	[16.38±1.74]
Total No. implantations	354	369	359	382
[Implantations/dam ± SD]	[15.39±2.41]	[16.04±1.61]	[15.61±1.67]	[15.92±1.47]
Total No. litters	23	23	23	24
Total No. live fetuses	337	347	340	362
[Live fetuses/dam ± SD]	[14.65±2.71]	[15.09±1.56]	[14.78±1.88]	[15.08±1.84]
Total No. dead fetuses	17	22	19	20
[Dead fetuses/dam]	[0.74]	[0.96]	[0.83]	[0.83]
Total No. resorptions				
Early	17	22	19	20
Late	0	0	0	0
Resorptions/dam				
Early	0.74	0.96	0.83	0.83
Late	0	0	0	0
Litters with total resorptions	0	0	0	0
Mean fetal weight (g ± SD)				
Males	3.68±0.24	3.71±0.17	3.67±0.23	3.45±0.20** (-6) [c]
Females	3.48±0.30	3.50±0.22	3.45±0.20	3.27±0.18* (-6)
Sex ratio (% male) [b]	49.55	52.45	52.06	50.28
Placental weight of live fetuses (g ± SD)				
Males	0.47±0.12	0.43±0.03	0.44±0.04	0.44±0.04
Females	0.48±0.21	0.41±0.03	0.42±0.04	0.42±0.04
Pre-implantation loss (%)	5.60	5.38	6.27	2.80
Post-implantation loss (%)	4.80	5.96	5.29	5.24

a Data obtained from page 53 in the study report.

b Calculated by the reviewer.

c Numbers in parentheses equal percent different from control; calculated by reviewer.

* Statistically different ($p<0.05$) from the control.

** Statistically different ($p<0.01$) from the control.

B. DEVELOPMENTAL TOXICITY:

1. **External examination:** Findings from the external examinations are given in Table 6a. Only two fetuses with external anomalies were observed: one fetus with cleft palate in the control group, and one runt in the 600 mg/kg bw/day group.

TABLE 6a. External examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Fetuses (litters) examined	337 (23)	347 (23)	340 (23)	362 (24)
No. Fetuses (litters) affected	1 (1)	0 (0)	0 (0)	1 (1)

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
Cleft palate[c]	1 (1)	0 (0)	0 (0)	0 (0)
Runt[c]	0 (0)	0 (0)	0 (0)	1 (1)

[a] Data obtained from pages 53 and 54 in the study report.

[b] Some observations may be grouped together.

[c] Fetal (litter) incidence

2. **Visceral examination:** Findings from the visceral examination are given in Table 6b. Ventricular septal defect was observed in one fetus from the control group, two fetuses from the 200 mg/kg bw/day group, and one fetus from the 600 mg/kg bw/day group. Dilatation of the renal pelvis was observed in 6 fetuses of the 50 mg/kg bw/day group (all unilateral) and in 2 fetuses of the 600 mg/kg bw/day group (one unilateral and one bilateral). Dilatation of the ureter was noted in 2, 7, 6, and 3 fetuses in the control, 50, 200, and 600 mg/kg bw/day groups, respectively. Of these, the increases in the incidences of unilateral dilation of the ureter in 5 and 6 fetuses of the 50 and 200 mg/kg bw/day groups, respectively, were statistically significant. Thymic remnants in the neck of fetuses in the 600 mg/kg bw/day group reached statistical significance (18.9% of the fetuses). The incidence was considerably above the historical control (6.3%) for the laboratory.

TABLE 6b. Visceral examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Fetuses examined	119	125	119	127
No. Fetuses affected	3	9	8	28**
Ventricular septal defect	1	0	2	1
Dilatation of the renal pelvis	0	6*	0	2
Unilateral	0	6*	0	1
Bilateral	0	0	0	1
Dilatation of the ureter	2	7	6	3
Unilateral	0	5*	6*	2
Bilateral	2	2	0	1
Thymic remnant in the neck	0	2	0	24**

[a] Data obtained from page 55 in the study report.
[b] Some observations may be grouped together.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

3. **Skeletal examination:** Findings from the skeletal examinations are given in Table 6c. The only skeletal anomaly observed was the fusion of the vertebral arch, which was observed in one fetus of the control group. Skeletal variations consisted of cervical ribs, lumbar ribs, splitting of the vertebral body, and an increase in pre-sacral vertebrae. The number of fetuses with skeletal variations showed a statistically significant and dose-related increase in fetuses of the 200 and 600 mg/kg bw/day groups. Cervical ribs were observed in 1 and 3 fetuses in the 200 and 600 mg/kg bw/day groups, respectively. Lumbar ribs were observed in 34, 44, 105, and 159 fetuses from the control and respective dose groups. The increases were dose-related and statistically significant in the 200 and 600 mg/kg bw/day groups. Split vertebral body was observed in 2, 2, 5, and 32 fetuses of the control and respective doses. The increases at 200 and 600 mg/kg bw/day were dose-related, and the increase at 600 mg/kg bw/day was statistically significant. A statistically significant increase in pre-sacral vertebrae was observed in the 600 mg/kg bw/day group. The concurrent control incidence of three of these

variations is within the historical control incidence [lumbar ribs (1.63-18.61%); split vertebrae (0-1.85%); pre-sacral vertebrae (0-2.6%)], but the incidences at 200 mg/kg/day and 600 mg/kg/day exceed that of the historical control (except pre-sacral vertebrae at 200 mg/kg/day).

TABLE 6c. Skeletal examinations (# fetuses (%) [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Fetuses examined	217	222	221	234
No. Fetuses with anomalies	1	0	0	0
Fusion of the vertebral arch	1	0	0	0
No. fetuses with variations	35	46	107**	173**
Cervical rib	0	0	1	3
Lumbar rib	34 (15.7%)	44 (19.8%)	105** (47.5%)	159** (67.9%)
Unilateral	22 (10.1%)	26 (11.7%)	52** (23.5%)	38** (16.2%)
Bilateral	12 (5.5%)	18 (8.1%)	53** (24%)	121** (51.7%)
Split vertebral body	2 (0.9%)	2 (0.9%)	5 (2.2%)	32** (13.7%)
Pre-sacral vertebra (increase)	3 (1.4%)	0 (0%)	3 (1.4%)	28** (12%)
No. fetuses with hypoplasia	0	0	0	1
Hypoplastic vertebral body	0	0	0	1

[a] Data obtained from page 56 in the study report.
[b] Some observations may be grouped together.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

On a litter basis (Table 6d), the incidence of these skeletal variations were increased at 200 ppm and 600 ppm (email submission dated August 7, 2014 from J. Asato for Sumitomo). Historical control were not provided on a litter basis.

Table 6d. Skeletal examination (litter incidence)[a]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Litters examined	23	23	23	24
Lumbar rib				
Unilateral	12 (52.2%)	13 (56.5%)	20 (87%)	20 (83.3%)
Bilateral	7 (30.4%)	8 (34.8%)	19 (82.6%)	23 (95.8%)
Split vertebral body	2 (8.7%)	2 (8.7%)	5 (21.7%)	16 (66.7%)
Pre-sacral vertebra (increase)	3 (13%)	0 (0%)	3 (13%)	13 (54.2%)

[a] Data obtained via email 8/7/2014

The study report included a summary of the progress of fetal ossification (Table 6e). In the 600 mg/kg bw/day group fetuses, there were significant increases in ossification of the lumbar vertebrae, along with decreases in the fifth and sixth sternebrae and in the proximal phalanges of the forelimb, both left and right.

TABLE 6e. Skeletal examinations [a] (progress of ossification)

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Fetuses examined	217	222	221	234
Vertebrae				
Lumbar arch right	6.01±0.03	6.01±0.00	6.01±0.03	6.12±0.15**
Lumbar arch left	6.01±0.03	6.01±0.00	6.01±0.03	6.12±0.15**
Lumbar arch body	6.01±0.03	6.01±0.00	6.01±0.03	6.12±0.15**
Sternebrae				
5th	179 (82.49)	169 (76.13)	169 (76.47)	165 (70.51)*
6th	210 (96.77)	206 (92.79)	202 (91.40)	197 (84.19)*

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
Phalanges of forelimb				
Proximal right	100 (9.22)	68[c] (6.13)	98 (8.95)	43 (3.68)
Proximal left	82 (7.56)	50 (4.50)	56 (5.07)	36 (3.08)

[a] Data obtained from page 57 in the study report.
[b] values of sternabrae, phalanges of forelimb represent the number of ossified and values in () represent ossification %.
[c] Illegible
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Effects on dams: In the 50 mg/kg bw/day group, no dose-related effects on the dams were obtained from general observation, body weight, body weight gain, food consumption during gestation, or from necropsy findings at the time of cesarean section. In the 200 mg/kg bw/day group, reduced body weight gain and reduced food consumption in the early period of administration were observed, but there were no dose-related effects on the dams during gestation or in the necropsy findings at the time of cesarean section. In the 600 mg/kg bw/day group, tremor or clonic convulsions were observed in the early period of administration in most F0 dams, accompanied by prone position in about half of the F0 dams and with staggered gait and incontinentia urinae in a few F0 dams. Two F0 dams were also observed with soiled perinaris, loose stool, salivation, lateral position, hypoactivity, and bradypnea; they died on days 9 and 12 of gestation. In the body weight, body weight gain, and food consumption of the 600 mg/kg group, low values were observed in the early period of administration. In the necropsy findings at the time of cesarean section, no change related to the test substance was observed in the dams except for increased blackish coloration and enlargement of the spleen found in one dam.

Effects on F1 fetuses: In the observations at the time of cesarean section, reduced body weight was observed in F1 fetuses in the 600 mg/kg bw/day group, and a slight growth retardation of the fetuses was recognized. There were no changes in the numbers of corpora lutea, implants, pre-implantation loss, early resorptions, late resorptions, dead fetuses, live fetuses, sex ratio, and placental weight of live fetuses in the treated groups. There were no effects on fetal lethality. A higher incidence of fetuses with skeletal variations was found in the 200 and 600 mg/kg bw/day groups. There was a higher incidence of lumbar rib in the 200 and 600 mg/kg bw/day groups, and a higher incidence of increased pre-sacral vertebrae and split vertebral body were observed in the 600 mg/kg bw/day group. Greater ossification of lumbar vertebrae and arch and lesser ossification of the 5^{th} and 6^{th} sternebrae were observed in the 600 mg/kg bw/day group. A higher incidence of a thymic remnant in the neck was observed in the 600 mg/kg group at the time of visceral examination. The no observed effect level was considered to be 50 mg/kg bw/day for both the dams and fetuses. It was concluded that S-41311 had no teratogenic potential under the condition of the present study.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Tremors, convulsions, and prone position were observed in most of the 600 mg/kg bw/day dams from GD 7-9. Two dams from the group that was allowed to litter died (GD 9 and GD 12). Body weights were comparable among the groups, although decreased body weight gains were observed initially (GD 6-10) at 200 and 600 mg/kg bw/day.

   The maternal LOAEL in rats treated with S-41311 on gestation days 6-17 was 600 mg/kg bw/day, based on deaths and clinical signs (tremors, convulsions, prone position). The NOAEL is 200 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment did not result in an increase in fetal deaths or resorptions.

   b. **Altered growth:** Mean fetal weight was significantly decreased in male and female fetuses in the 600 mg/kg bw/day group (↓6%). Additionally, in the 600 mg/kg bw/day group, there were decreases in the ossification percentages of the fifth and sixth sternebrae and the proximal phalanges of the forelimb (both left and right), providing additionally evidence of growth retardation at this dose. Significant increases in ossification of the lumbar vertebrae were observed at this dose also.

   c. **Developmental variations:** There was a significant increase in the number of fetuses with visceral variations in the 600 mg/kg bw/day group, particularly the fetuses with a thymic remnant. In the skeletal effects in fetuses, there was a dose-related increase in the number of skeletal variations starting at 50 mg/kg bw/day, and continuing to 200 and 600 mg/kg bw/day, where the effects were statistically significant. In the 600 mg/kg bw/day group fetuses, there were also significant increases in ossification of the lumbar vertebrae, and decreases in the ossification percentages of the fifth and sixth sternebrae and the proximal phalanges of the forelimb, both left and right. There was a dose-related increase in the incidence of litters with fetuses with unilateral and bilateral lumbar ribs and splitting of vertebral body.

   d. **Malformations:** There was no evidence of dose-related malformations.

      The developmental toxicity LOAEL is 200 mg/kg/day, based on an increase in fetuses and litters with unilateral and bilateral ribs. The developmental toxicity NOAEL is 50 mg/kg/day. At 600 mg/kg/day, there also was a decrease in fetal body weight and increased incidences of thymic remnant in the neck, pre-sacral vertebrae, and split vertebral body, and reduced ossification of 5th and 6th sternebrae and proximal forelimb phalanges.

3. **General comments about the overall study:**

   In reviewing this study,

   The food was pelleted. The investigator did not state how they accounted for spillage or if they covered the food with keepers.

C. STUDY DEFICIENCIES:

The reporting of the study procedures and results was not in accordance with OCSPP 870.3700 with respect to the following:

A. A major problem throughout the study is the allotment of males to the study groups. It is not known how the males were assigned among the three treatment and control groups; whether the males were represented in each group; duration of mating period.
B. The litter incidences for the visceral and skeletal findings were not available in the study report. Individual data were not provided. These data were requested and later submitted by the registrant *via* email (August 7, 2014).
C. Study procedures were not defined. SOPs were not included, Appendices were not available.
D. The animals were given well water through an automatic watering system or bottles, but it is not clear what determined the use of the watering system or the bottles.

The study is classified as A**cceptable/Guideline** and **it satisfies** the guideline requirement for a developmental toxicity study (OCSPP 870.3700) in the rat.

APPENDIX I: Reproduction Study

EXECUTIVE SUMMARY: In a combination reproduction and developmental toxicity study, S-41311 (Imiprothrin; 92.9% a.i.; Lot No. Y-011001) was administered to 9-12 female Sprague-Dawley strain of rats per dose by gavage in corn oil at dose levels of 0, 50, 200, or 600 mg/kg bw/day from gestation day 6 through day 17. On gestation day 20, these dams (specifically 12, 12, 11, and 9 dams from the 0, 50, 200, and 600 mg/kg bw/day groups, respectively) were allowed to litter and raise their offspring. The offspring were not treated. On delivery, the number of litters and number of live and dead offspring were measured; body weight, sex, and external anomalies of live offspring were examined. Offspring were weighed individually on days 0, 4, 7, 14, and 21 after birth. Postnatal differentiation of F1 offspring was examined based on pinna detachment on postnatal day 4, piliation on day 8, incisor eruption on day 10, gait and eyelid separation on day 15, and descent of testes on day 21. Function tests were carried out on righting and ipsilateral flexor reflex on postnatal day 5 and visual placing on day 16. When postnatal differentiation and functions were not completely developed on the designated day, the tests were continued until completion.

At weaning on postnatal day 22, one male and one female per litter were killed and examined for macroscopic organ and tissue changes. Skeletons were stained and examined for anomalies. After weaning, three male and three female offspring per litter were observed daily for clinical signs, and body weight and food consumption were measured weekly. In addition, these offspring were examined for auricle reflex on postnatal day 28, and for vaginal opening on postnatal day 42. Among these offspring, one male and one female per litter were selected for motor coordination (rotarod performance) at five weeks of age, water maze learning at six weeks of age, and open field behavior at eight weeks of age. The animals used for behavior and learning tests were sacrificed at 12 weeks of age, and organs and tissues were examined for macroscopic changes, and organ weights (heart, lungs, liver, kidneys, adrenals, brain, spleen, thymus, testes, and ovaries) were measured. *These organ weight data were not found in the study report*.

Under the conditions of this study, the offspring LOAEL, with dosing of the dams conducted on days 6-17 of gestation, is 600 mg/kg bw/day, based on an increase in the number of stillborn and an increased number of weanlings with lumbar ribs. The offspring NOAEL is 200 mg/kg bw/day.

The maternal LOAEL in rats treated with imiprothrin on gestation days 6-17 was 600 mg/kg bw/day, based on deaths and clinical signs (tremors, convulsions, prone position). The NOAEL is 200 mg/kg bw/day.

The reproductive toxicity portion of this study in the rat is classified as **Acceptable / Non-guideline.** The study is non-guideline because there was no pre-mating exposure period (only females dosed; only during GD 6-17), there was an insufficient number of dams per group (9-12 vs 20). Additionally, there are reporting deficiencies, in particular the omission of litter data and organ weight data and the inadequacy of the histopathology report. However, the study is acceptable because it provides some information on parameters in the offspring of dams exposed during gestation.

I. MATERIALS AND METHODS

A. MATERIALS: See main section of DER.

B. PROCEDURES AND STUDY DESIGN:

1. In-life dates: See main section of DER.

2. Mating: See main section of DER.

3. Animal assignment: See main section of DER. The number of males used in mating to produce the pregnant females dosed during gestation days 6-17 is not stated in the study report. The offspring (F1) animals were not treated.

4. Dose selection rationale: See main section of DER.

C. OBSERVATIONS:

1. Parental animals: For the F0 dams assigned to natural delivery, clinical observations were made on delivery and during lactation. Body weight and food consumption data were recorded on gestation days 0, 4, and 6-20, and on lactation days 0, 4, 7, 10, 14, 17, and 21. Dams were sacrificed after the pups were weaned. The animals were checked for mortality or clinical signs at least once daily.

2. Litter observations: The number of litters, and number of live and dead offspring were measured on delivery. Body weight, sex, and external anomalies of live offspring were examined. The number of live offspring per litter was adjusted to eight on postnatal day 4. The method used is not stated. If the litter contained less than eight offspring, all were retained. The live offspring were weighed individually on days 0, 4, 7, 14, and 21 after birth, and observed at least once daily for clinical signs. Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

Postnatal differentiation of F1 offspring was examined based on pinna detachment on postnatal day 4, piliation on day 8, incisor eruption on day 10, gait and eyelid separation on day 15, and descent of testis of day 21. Function tests were carried out on righting and ipsilateral flexor reflex on postnatal day 5 and visual placing on day 16. When postnatal differentiation and functions mentioned were not completely developed on the designated day, the tests were continued until completion.

At weaning on postnatal day 22, one male and one female per litter were sacrificed and examined for macroscopic organ and tissue changes. Skeletons were stained and examined for anomalies.

After weaning, three male and three female offspring per litter were observed daily for clinical signs, and body weight and food consumption were measured weekly. In addition, these offspring were examined for auricle reflex on postnatal day 28, and for vaginal opening on postnatal day 42. Among these offspring, one male and one female were selected for motor coordination (rotarod performance) at five weeks of age, water maze learning at six weeks of

age, and open field behavior at eight weeks of age. The animals used for behavior and learning tests were sacrificed at 12 weeks of age, and organs and tissues were examined for macroscopic changes. Necropsies were done on the animals sacrificed at 12 weeks of age. When the animals were sacrificed at 12 weeks of age, organ weights (heart, lungs, liver, kidneys, adrenals, brain, spleen, thymus, testes, and ovaries) were measured. These organ weight data were not found in the study report.

D. DATA ANALYSIS:

1. **Statistical analyses:** The mean values and standard deviations were calculated for the following items in each group: body weight, food consumption, duration of gestation, number of litters, total newborns, and live newborns, results of rotarod performance test, learning ability, and emotional examination. Bartlett's test was used to compare the variance among groups of data. When groups were accepted to be homogeneous, analysis of variance (ANOVA) was used for comparison of groups of data. When significant difference was shown among the groups, Dunnett's t-test (N equal to N) or Scheffe's test (N not equal to N) was finally required to determine the difference between control and treated groups. When the groups of data were heterogeneous in Bartlett's test, Kruskal-Wallis nonparametric ANOVA was used, When there was significant difference among the groups, Dunnett-type test (N equal to N) or Scheffe's test (N not equal to N) was required. Birth index, mating index, pregnancy index, sex ratio of live newborns were analyzed by chi-square test. The index of stillborn, external anomalies, skeletal anomalies and variations, live newborn, viability on day 4 or weaning, results of function test and postnatal differentiation were analyzed by Wilcoxon's test. The level of $P<0.05$ was considered to be significant in each test. Values of F1 offspring during lactation were treated with each litter as a unit.

2. **Indices:** The following indices were calculated:

   Reproductive Indices:

   Mating Index = (No. copulated/No. mated) X 100

   Pregnancy Index = (No. pregnant/No. copulated) X 100

   Gestation Index = (No. females with live newborns/No. pregnant females) X 100

   Birth Index = (No. live newborns/No. implants) X 100

   Offspring Viability Indices:

   Viability Index on Day 4 = (No. alive on day 4/No. of liveborn) X 100

   Weaning Index = (No. of live weanlings/No. of liveborn after culling) X 100

3. **Historical control data:** Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** Deaths of two females in the 600 mg/kg bw/day group were attributed to the test compound (GD 9 and GD 12). The death of the third female was presumably due to a dosing error. In the necropsy at weaning and in the F1 offspring sacrificed at 12 weeks of age, there were no treatment-related anomalies. There was a slight decrease in the gestation index (not significant) and an increase in the number of stillborn animals in the 600 mg/kg bw/day group.

TABLE 1. Necropsy findings of dead dams [a]

Observation	Dose in mg/kg bw/day			
	Control	50	200	600
Partial dark coloration in the lung and retention of frothy fluid in the trachea	0	0	0	1
Atrophy of thymus and partial light gray coloration in liver	0	0	0	1
Black red macules in lung, ulcer, black red spot in esophagus, and retention of bloody fluid in the thoracic cavity (death due to administration error)	0	0	0	1

[a] Data obtained from pages 21 in the study report.

2. **Body weight:** Body weight data are summarized in Table 2. There were no statistically significant differences on any day and no dose-related change in body weight during lactation.

TABLE 2. Mean (±SD) maternal body weight and body weight gain during lactation (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (12)[c]	50 (12)	200 (11)	600 (9)
Maternal wt. at lactation day 0	297.2±15.8	298.7±19.7	293.5±13.4	295.8±14.7
Maternal wt. at lactation day 21	323.4±18.1	319.0±18.0	317.6±12.2	330.6±14.8
Wt. gain, lactation days 0-21 [b]	26.2	20.3	24.1	34.8

[a] Data obtained from page 47 in the study report.

[b] Calculated by the reviewer using group means; not analyzed statistically.

[c] n=11 on LD 0

3. **Food consumption:** Food consumption data were reported for lactation days 1, 4, 7, 10, 14, 17, and 21. There were no statistically significant changes in food consumption during the reported days. Total food consumption during lactation was not reported. Food consumption during three specific days of lactation is shown in Table 3 below. In the 600 mg/kg group, there was more food consumed than in the control or the two other treatment groups, but the differences were not significant.

TABLE 3. Mean maternal food consumption (g±SD) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (12)	50 (12)	200 (11)	600 (9)
Lactation day 7	43.6±6.8	42.8±3.1	45.2±4.6	46.9±6.0

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (12)	50 (12)	200 (11)	600 (9)
Lactation day 14	54.3±9.3	56.9±4.2	56.9±5.2	59.7±4.3
Lactation day 21	67.1±11.1	67.0±7.6	69.9±6.1	73.2±6.2

[a] Data obtained from page 50 in the study report.

4. **Gross pathology:** There were no treatment-related gross findings at necropsy.

5. **Reproduction study data:** Observations at birth are summarized Table 4. Gestation index was decreased in the 600 mg/kg bw/day group (82% vs 100% in control and other dose groups). The number of stillborn males and females was increased in the 600 mg/kg bw/day group (8% of control litters vs 33% of 600 mg/kg/day litters). The number of implantations, number of live offspring, and sex ratio showed no dose related changes (Table 5). Growth observations of F1 offspring from birth to weaning are summarized in Table 6. No dose-related differences were observed in growth of offspring to weaning. There were no deaths in the offspring between lactation days 5 and 22. Body weight gain from birth to weaning is summarized in Table 7. In both males and females, there was a slight increase in body weight gain during the time to weaning in the 600 mg/kg bw/day group. Growth observations post-weaning to day 84 are summarized in Table 8. In males, weight gain increased in a dose-related manner. This gain is possibly attributable to increased food consumption on lactation days 56 to 70.

Table 4. F1 offspring from F0 dams

Observation	Dose in mg/kg bw/day			
	Control	50	200	600
Gestation index (%)[A]	100	100	100	81.8
# stillborn	1M	1M, 1F	1M	3M, 3F, 1C[B]

Data obtained from Table 12, page 58 in the study report. [A]# females with liveborn/# pregnant dams x 100; [B]C (cannibalized);

TABLE 5. Reproduction study observations at birth [a]

Observation	Dose (mg/kg bw/day)			
	Control	50	200	600
# Animals assigned (mated)	12	12	11	11
# Animals with live offspring	12	12	11	9
Gestation Index [b]	100	100	100	81.82
Gestational days (± SD)	21.75±0.45	21.67±0.49	21.91±0.30	22.00±0.00
No. implantations (No. /litter ± SD)	177 (14.75±4.05)	182 (15.17±1.75)	165 (15.00±1.67)	141.0 (15.67±1.12)
Total no. offspring (No./litter ± SD)	172 (14.33±3.94)	178 (14.83±1.47)	158 (14.36±1.80)	136 (15.11±1.05) [e]
Male	86 (7.16)	81 (6.75)	83 (7.55)	66 (7.33)
Female	86 (7.16)	97 (8.08)	75 (6.52)	69 (7.67)
No. live offspring (No./litter ± SD)	171 (14.25±4.00)	176 (14.67±1.44)	157 (14.27±1.85)	129 (14.33±1.32)
Male	85 (7.08)	80 (6.67)	82 (7.45)	63 (7.0)
Female	86 (7.16)	96 (8.0)	75 (6.82)	66 (7.33)
No. stillborn (% fetuses)	1 (0.58)	2 (1.12)	1 (0.63)	7 (5.15) [e]
Male	1	1	1	3
Female	0	1	0	3
Litters with stillborn (% litters)[f]	1 (8.3%)	2 (16.7%)	1 (9.1%)	3 (33.3%)
Birth Index [c]	96.61	96.70	95.15	91.49
Sex ratio live offspring (% male) [d]	49.71	45.45	52.23	48.84
Mean wt. live offspring (± SD) [e]				
Male	6.3±0.7	6.2±0.4	6.5±0.4	6.6±0.4
Female	5.9±0.7	5.8±0.4	6.1±0.3	6.2±0.4

a Data obtained from pages 58, 59 in the study report.

b Gestation Index = (No. of females with live newborns/No. of pregnant females) X 100

c Birth Index = (No. live offspring/No. implantations) X 100

d % male calculated by the reviewer

e Includes 1 cannibalized animal (sex not reported)

f email submission (8/2014)

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

TABLE 6. Reproduction study – observations of F1 offspring on days 4-22 [a,b]

Observation	Dose (mg/kg bw/day)			
	Control	50	200	600
Neonatal period (birth to day 4)				
No. live born	171 (14.25)	176 (14.67)	157 (14.27)	129 (14.33)
Male	85 (7.08)	80 (6.67)	82 (7.45)	63 (7.0)
Female	86 (7.16)	96 (8.0)	75 (6.82)	66 (7.33)
No. alive on day 4	169 (14.08)	174 (14.5)	156 (14.18)	126 (14.0)
Male	85 (7.08)	79 (6.38)	82 (7.45)	62 (6.88)
Female	84 (7.0)	95 (7.92)	74 (6.73)	64 (7.11)
No. culled on day 4	78 (6.50)	78 (6.50)	68 (6.18)	54 (6.00)
Male	39 (3.25)	32 (2.67)	38 (3.45)	25 (2.78)
Female	39 (3.25)	46 (3.83)	30 (2.73)	29 (3.22)
No. alive on day 4 after culling	91 (7.58)	96 (8.00)	88 (8.00)	72 (8.00)
Male	46 (3.83)	47 (3.92)	44 (4.00)	37 (4.11)
Female	45 (3.75)	49 (4.08)	44 (4.00)	35 (3.89)
Viability Index on Day 4	98.83	98.86	99.36	97.67
Males	100	98.75	100	98.41
Females	97.67	98.96	98.67	96.97
No. alive on day 22	91 (7.58)	96 (6.50)	88 (8.00)	72 (8.00)
Male	46 (3.83)	47 (2.67)	44 (4.00)	37 (4.11)
Female	45 (3.75)	49 (3.83)	44 (4.00)	35 (3.89)
Weaning Index	100	100	100	100
Males	100	100	100	100
Females	100	100	100	100
No. deaths on days 5-22	0	0	0	0
No. deaths post-weaning	0	0	1 (male)	0

a Data obtained from page 59 in the study report.

b No./litter (in parentheses) calculated by the reviewer

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

TABLE 7. Reproduction study – Body Weight/growth observations to weaning (g ± SD) of F1 offspring [a]

Observation (body weight)	Dose (mg/kg bw/day)			
	Control	50	200	600
Male - Day 0	6.3±0.7	6.2±0.4	6.5±0.4	6.6±0.4
Day 4	10.0±1.9	9.4±0.8	10.0±1.1	10.3±0.9
Day 7	16.3±2.1	15.6±1.1	16.4±2.0	17.0±1.3
Day 14	34.3±2.5	33.6±1.7	33.5±2.9	35.1±2.8
Day 21	54.4±4.3	53.8±3.7	54.1±4.2	55.6±4.8
Wt. gain – days 0-21 [b]	48.1	47.6	47.6	49.0
Female – Day 0	5.9±0.7	5.8±0.4	6.1±0.3	6.2±0.4
Day 4	9.4±1.7	8.9±0.8	9.4±1.1	10.0±1.0
Day 7	15.5±2.3	14.9±1.0	15.3±1.8	16.4±1.6
Day 14	33.1±3.0	32.5±1.6	31.9±2.4	34.1±3.1
Day 21	52.4±4.4	52.4±3.3	51.3±3.7	54.4±5.3
Wt. gain – days 0-21 [b]	46.5	46.6	45.2	48.2

a Data obtained from pages 60, 61 in the study report.

b Calculated by the reviewer

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

TABLE 8. Reproduction study – growth observations post-weaning (g ± SD) of F1 offspring [a]

Observation (body weight)	Dose (mg/kg bw/day)			
	Control	50	200	600
No. males	34	36	33	27
Male - Day 28	96.8±8.4	96.0±8.7	95.1±7.9	98.5±8.9
Day 35	158.9±14.2	157.9±13.9	156.6±12.9	162.5±14.7
Day 42	226.1±18.4	227.3±18.4	224.7±18.7	232.9±19.4
Day 49	284.5±21.7	287.5±24.5	284.8±20.4	294.3±23.1
Day 56	342.1±25.7	347.0±29.5	344.0±23.3	355.8±25.9
Day 63	385.3±27.9	390.0±34.0	388.5±24.1	402.0±26.7
Day 70	423.8±32.9	427.3±38.6	428.2±26.4	443.0±31.0
Day 77	455.0±35.9	459.1±40.9	461.5±28.3	476.8±34.5
Day 84	481.3±38.5	487.1±43.9	491.5±32.0	507.7±38.1
Wt. gain – days 28-84 [b]	384.5	391.1	396.4	409.2
No. females	35	36	33	27
Female – Day 28	87.6±7.6	88.7±6.3	85.1±6.3	88.4±7.6
Day 35	134.0±12.8	136.7±8.9	132.2±9.1	135.1±11.3
Day 42	171.7±16.6	176.5±11.9	172.8±13.9	172.5±13.7
Day 49	194.0±18.9	200.8±15.1	196.8±15.3	198.1±17.3
Day 56	216.6±23.1	226.6±18.5	221.9±17.7	222.6±18.6
Day 63	235.3±26.2	247.0±20.8	242.1±19.8	241.9±20.2
Day 70	252.3±30.2	263.1±25.2	259.5±23.2	259.4±25.3
Day 77	263.8±30.6	274.2±26.8	272.2±26.4	272.4±25.7
Day 84	274.1±30.1	286.2±28.5	284.8±27.0	285.0±26.7
Wt. gain – days 28-84 [b]	186.5	197.5	199.7	196.6

a Data obtained from pages 62, 63 in the study report.

b Calculated by the reviewer

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

Postnatal differentiation of the F1 offspring, male and female showed no significant changes in pinna detachment, piliation, incisor eruption, eyelid separation, gait, descent of testis, or vaginal opening (Table 9). Function tests done in the offspring (righting reflex, ipsilateral flexor reflex, visual placing, and Preyer's reflex) showed no difference between control and treated groups (Table 10).

There were no differences between groups in the rotarod performance test and in the water maze test. In the open field behavior test, there was an increased amount of grooming in the 50 mg/kg bw/day males, and decreased urination in the 50 and 600 mg/kg bw/day males. No differences between control and treated females were found in the open-field behavior test.

TABLE 9. [a] Postnatal Differentiation of F1 Offspring of F0 Dams				
Observation	**Dose group (mg/kg/day)**			
	0	**50**	**200**	**600**
MALES				
Pinna detachment (4 days)	85/85 (100)	79/7 (100)	82/82 (100)	62/62 (100)
Piliation (8 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Incisor eruption				
10 days	36/46 (78.26)	37/47 (78.72)	40/44 (90.91)	35/37 (94.59)
11 days	43/46 (93.48)	45/47 (96.74)	44/44 (100)	37/37 (100)
12 days	46/46 (100)	47/47 (100)	-	-
Eyelid separation				
15 days	42/46 (91.3)	43/47 (91.49)	44/44 (100)	32/37 (80.49)
16 days	46/46 (100)	47/47 (100)	-	37/37 (100)
Gait (15 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Descensus testis				
21 days	46/46 (100)	45/47 (95.47)	42/44 (95.45)	37/37 (100)
22 days	-	47/47 (100)	44/44 (100)	-
FEMALES				
Pinna detachment (4 days)	84/84 (100)	95/95 (100)	73/73 (100)	64/64 (100)
Piliation (8 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)
Incisor eruption				
10 days	32/45 (71.11)	35/49 (71.43)	40/44 (90.91)	30/35 (85.71)
11 days	38/45 (86.67)	45/49 (91.84)	43/44 (97.73)	35/35 (100)
12 days	45/45 (100)	49/49 (100)	44/44 (100)	-
Eyelid separation				
15 days	43/45 (95.56)	46/49 (93.88)	44/44 (100)	35/35 (100)
16 days	45/45 (100)	49/49 (100)	-	-
Gait (15 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)
Vaginal opening (42 days)	35/35 (100)	36/36 (100)	44/44 (100)	35/35 (100)

[a] Data obtained from Table 20, page 66 in the study report.

TABLE 10. [a] Function Tests of F1 Offspring of F0 Dams				
Observation	**Dose group (mg/kg/day)**			
	0	**50**	**200**	**600**
MALES				
Righting reflex				
5 days	41/46 (80.13)	43/47 (91.49)	43/44 (97.73)	35/37 (94.59)
6 days	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Ipsilateral flexor reflex (5 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Visual placing (16 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Preyer's reflex 500 Hz (60 dB) (28 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
Preyer's reflex 2000 Hz (60 dB) (28 days)	46/46 (100)	47/47 (100)	44/44 (100)	37/37 (100)
FEMALES				
Righting reflex				
5 days	42/45 (93.33)	42/49 (85.71)	41/44 (93.18)	31/35 (88.57)
6 days	45/45 (100)	49/49 (100)	43/44 (97.73)	34/35 (97.14)
7 days	-	-	44/44 (100)	35/35 (100)
Ipsilateral flexor reflex (5 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)
Visual placing (16 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)
Preyer's reflex 500 Hz (60 dB) (28 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)
Preyer's reflex 2000 Hz 60 (dB) (28 days)	45/45 (100)	49/49 (100)	44/44 (100)	35/35 (100)

[a] Data obtained from Table 21, page 67 in the study report.

At weaning, one male and one female from each litter were stained and examined for skeletal alterations; these results are summarized in Table 11. Male and female offspring results were combined, but litter incidence was not provided in the study report (submitted *via* email

subsequently). There was a significant increase in the number of offspring with bilateral lumbar ribs in the 600 mg/kg bw/day group.

TABLE 11. Skeletal examinations of weanling F1 males and females [a,b]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
#Fetuses (litters) examined	22 (11)	24 (12)	22 (11)	18 (9)
#Fetuses with skeletal anomalies	0	0	0	0
#Fetuses with skeletal variations	1	0	3	8**
Lumbar rib	1	0	3	8**
Unilateral	1 [1, 9%][c]	0	2 [2, 18%]	1 [1, 11%]
Bilateral	0	0	1 [1, 9%]	7** [5, 56%]
Lumbarization	0	0	0	1
Pre-sacral vertebrae (increase)	0	0	1 [1, 9%]	2 [1, 11%]

a Data obtained from pages 70 in the study report.

b Litter incidence was not reported, except for overall examination (email submission 8/2014).

[c] [# litters affected, %]

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Gestation index was lower in the 600 mg/kg bw/day group due to the death of two dams. The same higher incidence of lumbar ribs as seen in the F1 fetuses was also observed during the skeletal examination of the F1 offspring at weaning. There were no dose-related effects in postnatal viability, developmental differentiation, sensory function, motor coordination, learning ability, or emotional behavior. The no-observed-effect level was considered to be 200 mg/kg bw/day for the offspring under the conditions of this study.

B. REVIEWER COMMENTS:

This non-guideline reproductive toxicity study in the rat was not designed to fulfill OCSPP 870.3800 and does not do so. The study lacks robustness because of the small number of animals used in each dose, many of the required parameters were not evaluated, and the dosing schedule was inappropriate for a reproduction study. However, the study does provide useful supplemental information about offspring effects.

Under the conditions of this study, the offspring LOAEL is 600 mg/kg bw/day, based on an increase in the number of stillborn and an increased number of weanlings with lumbar ribs. The offspring NOAEL is 200 mg/kg bw/day.

C. STUDY DEFICIENCIES:

Although the investigator states in the statistical analysis section that the F1 data were analyzed with the litter as a unit, the litter data are not always reported in the tables.

According to the investigator, some organs were weighed, but the weights were not found in the study report.

The histopathology reported was insufficient.

APPENDIX II. Developmental Toxicity Study of F1 Animals

EXECUTIVE SUMMARY: This was a non-guideline second-generation part of a combination developmental toxicity/reproduction effects study in rats. F1 animals from F0 dams treated during GD 6-17 were mated at 12-13 weeks of age to produce offspring that were assessed for developmental toxicity effects on gestation day 20 (GD 20). The F1 females were not treated with the test material. On GD 20, caesarean sections were performed on the untreated F1 pregnant dams, and corpora lutea, implants, pre-implantation loss, live and dead fetuses, sex ratio, body weight, and placental weight were recorded. The fetuses were examined for external anomalies but not for skeletal or visceral anomalies.

The maternal NOAEL for F1 females dosed *in utero* only (GD 6-17) and not following birth, growth, or during their pregnancy, is 600 mg/kg bw/day, based on lack of effect at the high dose.

Under the conditions of this study (no skeletal or visceral examinations), the developmental NOAEL for offspring of dams dosed *in utero* only (GD 6-17) and not following birth, growth, or during their pregnancy is 600 mg/kg bw/day, based on lack of effect at the high dose.

The developmental toxicity study in the rat is classified **Acceptable / Non-guideline.** It is non-guideline because it does not follow any guideline requirement; however, it supplements the main part of the study and provides limited information of offspring from dams exposed *in utero* to the test material. The same basic reporting deficiencies seen in the main guideline study and the reproductive part reported in Appendix I were found in this phase of the study report also.

I. MATERIALS AND METHODS

A. MATERIALS: See main section of this DER. These F1 Sprague-Dawley rats were not treated with the test material; they are the offspring of dams treated during GD 6-17.

B. PROCEDURES AND STUDY DESIGN

1. **In-life dates:** Not stated in the study report.

2. **Mating:** At 12-13 weeks of age, F1 males and females were mated 1:1 exclusive of siblings. The first mating was for two weeks. A second mating was done for females in the control, 50 mg/kg bw/day, and 200 mg/kg bw/day groups that did not copulate during the first mating (Table 1). Confirmation of mating was determined by a vaginal plug or the presence of spermatozoa in the vaginal smear, and was designated as day [0] of gestation.

TABLE 1. Mating and pregnancy of F1 offspring

Treatment group	Control	Low-Dose	Mid-Dose	High-Dose
Dose (mg/kg bw/day)	0	50	200	600
First mating:				
No. males mated	23	24	21	18
No. females mated	23	24	21	18
No. males copulated	22	23	20	18
No. females copulated	22	23	20	18
No. males that impregnated	22	21	20	16
No. females pregnant	22	21	20	16
Second mating:				
No. of males mated	1	1	1	0
No. females mated	2	1	2	0
No. males copulated	1	1	1	0
No. females copulated	2	1	2	0
No. males that impregnated	1	1	0	0
No. females pregnant	2	1	2	0
Total mated:				
No. males mated	23	24	21	18
No. females mated	24	24	22	18
No. males copulated	23	24	21	18
No. females copulated	24	24	22	18
No. males that impregnated	23	22	20	16
No. females pregnant	24	22	22	16
Copulation Index – females [b]	24/24 (100%)	24/24 (100%)	22/22 (100%)	18/18 (100%)
Pregnancy Index – Females [c]	23/24 (95.89%)	22/24 (95.89%)	20/22 (90.91%)	16/18 (95.24%)

a Data obtained from page 76 in the study report. Some of the males were mated with non-treated females and some females were mated with previously copulated males.

b Copulation Index = (No. copulated/No. Mated) X 100

c Pregnancy Index = (No. pregnant/No. copulated) X 100

3. <u>Animal assignment</u>: The method of animal assignment was not described.

4. <u>Dose selection rationale</u>: The F1 animals used in this study were the offspring of treated animals. See the main section of this DER for rationale.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** Duration of mating, mating index, and pregnancy index were calculated. The animals were checked for mortality or clinical signs at least once daily. Body weight and food consumption were recorded on gestation days 0, 4, 7, 10, 14, 17, and 20. Dams were sacrificed on gestation day 20. After gross examination of the organs and tissues, the ovaries and uterus were removed and the numbers of corpora lutea, implants, early resorptions, late resorptions, dead fetuses, and live fetuses were counted. The copulated but not pregnant females were sacrificed on day 20 after copulation and the males with confirmed mating were sacrificed after the mating period. They were examined for macroscopic changes of the organs and tissues. The sexual organs (testis, epididymis, prostate, or ovary and uterus) of males and females which copulated but did not impregnate or were not pregnant were examined for histopathological changes. The abnormal organs and tissues of males and females observed at necropsy were fixed in 10% neutral buffered formalin. Histopathological summaries were not provided in the study report. The animals that died during the study were necropsied immediately and gross observations were made. The abnormal organs and tissues were fixed in 10% neutral buffered formalin, and if necessary were examined histopathologically.

2. **Fetal evaluations:** The live fetuses and placentas were weighed, and live fetuses were sexed and examined for external anomalies including the oral cavity. Fetuses with external anomalies were fixed in 10% neutral buffered formalin. The fetuses were not examined for skeletal or visceral anomalies.

D. DATA ANALYSIS:

1. **Statistical analyses:** Mean values and standard deviations were calculated for each group for body weight, food consumption, and numbers of implantation sites, litters, corpora lutea, implants, and live fetuses. Bartlett's test was used to compare the variance among groups of data. When groups were accepted to be homogeneous, analysis of variance (ANOVA) was used for comparison of groups of data. When significant difference was shown among the groups, Dunnett's test (N equal to N) or Scheffe's test (N not equal to N) was finally required to determine the difference between control and treated groups. When the groups of data were heterogeneous in Bartlett's test, Kruskal-Wallis non-parametric ANOVA was used. When there was significant difference among the groups, Dunnett-type test or Scheffe's test was required. Mating index, pregnancy index, and sex ratio of live fetuses were analyzed by chi-square test. External anomalies, visceral anomalies, skeletal anomalies, skeletal variations, delayed ossification, progress of ossification, pre-implantation loss, early resorptions, late resorptions, and dead fetuses were analyzed by Wilcoxon's test. In each test, a level of $P<0.05$ was considered statistically significant. Values of F1 fetuses were treated with each litter as a unit. This information was taken from the report, pages 18 and 19.

2. **Indices:**

   Copulation Index = (No. copulated/No. mated) X 100

   Pregnancy Index = (No. pregnant/No. copulated) X 100

Formulas for pre-implantation and post-implantation loss were not provided.

3. **Historical control data:** Historical control data were provided in a very limited amount to allow comparison with concurrent controls. Laboratory weights for male and female fetuses were provided for F1 animals (3.57-3.79 g for males; 3.38-3.65 g for females), but the number of litters and the number of fetuses represented were not stated.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** No clinical signs or deaths were reported.

2. **Body weight:** Body weight data are summarized in Table 2. No statistically significant differences were reported at any time during gestation. Body weight gain during gestation was lower in the 600 mg/kg group than in the control or other treated groups.

TABLE 2. Mean (±SD) F1 maternal body weight and body weight gain during gestation (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (24)	50 (24)	200 (22)	600 (18)
Day 0 body weight	280.5±30.2	294.8±29.4	290.3±31.4	289.7±23.0
Day 20 body weight	424.2±37.2	436.4±52.6	437.1±40.9	418.8±37.3 ↓2%
Body weight gain [b]	143.7	141.6	146.8	129.1 ↓10%

[a] Data obtained from page 77 in the study report.

[b] Calculated by reviewer from group means; not analyzed statistically.

3. **Food consumption:** Food consumption data for these animals are not described in the study report.

4. **Gross pathology:** In the pregnant dams there were no significant differences in general condition and body weight change during gestation.

5. **Cesarean section data:** Cesarean section data are summarized in Table 3. Two non-pregnant females were observed in each of the 50 and 600 mg/kg bw/day groups. The number of corpora lutea per dam and the number of implantations per dam were slightly less in the 200 and 600 mg/kg bw/day groups than in the control and 50 mg/kg bw/day group, but the decreases were not dose-related. There was no effect on the number of live fetuses per litter, number of dead fetuses per litter, mean fetal weight, placental weight, and pre-implantation loss. Post-implantation loss was less in the 600 mg/kg bw/day group than in any other group. Mean fetal weight of male and female fetuses was within the limits of background data of the laboratory (3.57-3.79 g for males; 3.38-3.65 g for females).

TABLE 3. Cesarean section observations [a, b]

Observation	Dose (mg/kg bw/day)			
	0	50	200	600
# Animals assigned (mated)	24	24	22	18
# Animals pregnant	24	22	22	16
Pregnancy rate (%)	100	91.67	100	88.89
# Non-pregnant	0	2	0	2
Maternal wastage	N/A	N/A	N/A	N/A
Total No. corpora lutea Corpora lutea/dam [b]	393 16.38±2.50	359 16.32±1.25	342 15.55±2.52	255 15.94±1.69
Total No. implantations Implantations/dam[b]	364 15.17±1.40	342 15.55±1.26	323 14.68±3.05	236 14.75±2.35
Total No. litters	24	22	22	16
Total No. live fetuses Live fetuses/dam[b]	343 14.29±2.10	317 14.41±1.84	302 13.73±2.83	228 14.25±2.29
Total No. dead fetuses (Dead fetuses/dam)	21(0.87)	25(1.14)	21(0.95)	8(0.50)
Total No. resorptions	21	25	21	8
Early	21	25	21	8
Late	0	0	0	0
Resorptions/dam	0.87	1.14	0.95	0.50
Litters with total resorptions	N/A	N/A	N/A	N/A
Mean fetal weight (g±SD)				
Males	3.63±0.27	3.77±0.28	3.72±0.24	3.75±0.27
Females	3.41±0.25	3.55±0.28	3.49±0.26	3.49±0.26
Sex ratio (% male)[c]	52.19	46.69	52.32	49.12
Placental weight of live fetuses (g±SD)				
Males	0.45±0.05	0.45±0.04	0.45±0.05	0.43±0.03
Females	0.44±0.04	0.45±0.04	0.44±0.08	0.43±0.04
Pre-implantation loss (%)	29(7.38)	17(4.74)	19(5.56)	19(7.45)
Post-implantation loss (%)	21(5.77)	25(7.31)	21(6.56)	8(3.39)

a Data obtained from pages 76, 80 in the study report.

b Data are reported as Mean ± SD, where appropriate.

c Calculated by the reviewer

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

B. DEVELOPMENTAL TOXICITY:

1. **External examination:** Findings from the external examinations of the F2 fetuses are given in Table 4. All live fetuses were examined for external anomalies. From the control group, one fetus was reported with edema and anal atresia. From the 200 mg/kg BW/day group, one runt was reported. The laboratory's definition of runt was not stated.

2. **Visceral examination:** Visceral examination was not done.

3. **Skeletal examination:** Skeletal examination was not done.

TABLE 4. External examinations of F2 fetuses [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	50	200	600
No. Fetuses (litters) examined	343 (24)	317 (22)	302 (22)	228 (16)
No. Fetuses (litters) affected	1 (1) [c]	0	1 (1)	0
Edema and anal atresia	1 (1)	0	0	0
Runt	0	0	1 (1)	0

[a] Data obtained from pages 81 in the study report.

[b] Some observations may be grouped together.

[c] Fetal (litter) incidence

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

In the F1 reproductive ability, there were no changes related to the test substance in the copulation index, pregnant index, and the duration of mating in the treated groups. During the gestation period, there were no changes in the general condition and in the body weight of F1 pregnant rats. There were no dose-related changes in the numbers of corpora lutea, implants, pre-implantation loss, early resorptions, late resorptions, dead fetuses, live fetuses, sex ratio, body weight, and placental weight of live fetuses. In the external examination of F2 fetuses, edema and anal atresia were found in one F2 fetus in the control group and one runt among the F2 fetuses in the 200 mg/kg bw/day group.

The no-observed-effect level was considered to be 50 mg/kg bw/day for both dams and fetuses. The compound had no teratogenic potential under the condition of the present study.

B. REVIEWER COMMENTS:

The investigator's statement concerning the 50 mg/kg bw/day NOAEL may have applied to the entire (three-part) study and not strictly to the developmental toxicity study of F1 pups. This is difficult to determine. Obviously, the reviewer's NOAEL for the F1 developmental toxicity study is higher. There were no observed effects at 600 mg/kg bw/day in the abbreviated developmental toxicity done on F1 mated animals.

1. **Maternal toxicity:** There were no observed effects on F1 dams who were not dosed directly. **The maternal NOAEL is 600 mg/kg bw/day, based on lack of effect at the high dose.**

2. **Developmental toxicity: Under the conditions of this study (no skeletal or visceral examinations), the developmental (external examinations only) NOAEL is 600 mg/kg bw/day, based on lack of effects on parameters monitored at the high dose.**

C. STUDY DEFICIENCIES:

In this study, there was a problem in tracking the mating of the males and females.

It would seem appropriate, based on the increases in skeletal variations noted in the F1 fetal and weanling offspring, that the F2 fetal skeletons should have been examined.